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Accumulation of Inorganic and Methylmercury by Freshwater Phytoplankton in Two Contrasting Water Bodies

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Phytoplankton concentrate mercury from their aqueous surroundings and represent the primary entry point for Hg in aquatic food webs. We used ^{203}Hg to compare the uptake of inorganic mercury, Hg(II), and methylmercury, MeHg, in four phytoplankton species (a diatom, a chlorophyte, a cryptophyte, and a cyanobacterium) in two waters containing different concentrations of dissolved organic carbon (DOC). At steady state, volume concentration factors (VCFs) for Hg(II) in the four species were similar and ranged from 0.5 to 5 $\times$ 10^4 for both water types, whereas VCFs for MeHg exceeded those for Hg(II) and ranged from 1.3 to 14.6 $\times$ 10^5. The VCFs for MeHg in the three eukaryotic cells in the high DOC water were 2−2.6 times greater than those in the low DOC water, but the VCFs for the prokaryote were similar in both waters. Higher cell surface area to volume ratios correlated with increased MeHg concentrations but not with Hg(II). In both water types, VCFs of Hg(II) were similar for living and heat-killed cells, but the VCFs of MeHg were 1.5−5.0 times greater in living cells, suggesting an active uptake component for MeHg. Hg(II) and MeHg were entirely bound to cell surfaces of the dead cells, whereas 59−64% of the MeHg and 9−16% of the Hg(II) in living cells entered the cytoplasm.

Introduction

The accurate measurement of accumulated metal concentrations in biota at the base of aquatic food webs is important for both field and laboratory based ecological risk assessment (*1*). Because most of the mercury that accumulates in higher trophic level species originates from consumed prey rather than direct, aqueous accumulation (*2*, *3*), characterizing the partitioning of mercury from water to phytoplankton is critical. The bioconcentration of both inorganic and methylmercury (MeHg) has been examined at the base of aquatic food webs (*4*, *5*). As with other metals, the greatest bioconcentration step for Hg in aquatic food chains occurs at the base (bacteria and phytoplankton), resulting from the partitioning of Hg from the aqueous phase to these cells (*6*−*8*). Relative to ambient water, phytoplankton cells typically have Hg concentrations that are over ten thousand times greater and thus represent an enriched source of Hg for the rest of the food chain. The bioaccumulation of MeHg in particular has important implications for its trophic transfer in food chains because of its tendency to biomagnify with trophic levels beyond phytoplankton (*5*, *9*). As fundamental contributors to the base of aquatic food webs, species-specific differences in mercury accumulation dynamics or varying partitioning between unique natural waters have important consequences for a watershed's susceptibility to produce fish with Hg concentrations exceeding consumption advisory levels (*10*, *11*). An important aspect of bioaccumulation is the degree to which Hg(II) and MeHg interact with dissolved organic matter (*12*, *13*) affecting the concentration factors between phytoplankton and other biota and their aqueous surroundings (*14*, *15*). Fish and higher trophic levels obtain most of their Hg from dietary sources, so even small differences in concentration factors at the base of aquatic food webs should have measurable consequences on the overall biomagnification of Hg in a given watershed.

Inorganic Hg(II) and MeHg in surface waters are normally complexed with naturally occurring, dissolved organic carbon (DOC) (*7*, *16*−*18*). Furthermore, the overall effect of mercury−organic matter complexation on bioaccumulation in phytoplankton and higher trophic levels is unclear. Previous research suggests inhibitory effects of DOC complexation on cell accumulation of Hg in phytoplankton (*13*, *19*) but no measurable effects on fish accumulation of Hg (*20*). Other studies with phytoplankton showed enhanced uptake of Al and Cd in the presence of DOC, attributing this to DOC-enhanced membrane permeability for these metals (*14*, *21*). Whether there is an increased ability of Hg to cross living membranes in cells exposed to DOC, as observed for Al and Cd, remains unclear. There is a clear need for mechanistic studies examining Hg accumulation dynamics into biota at the base of aquatic food chains in the presence of natural DOC.

An ideal system for investigating the effects of water chemistry and phytoplankton species composition on Hg accumulation dynamics is the San Francisco (SF) Bay watershed in California which includes the Central Delta fed by the Sacramento and San Joaquin rivers and a long history of Hg contamination (*21*, *22*). The Cosumnes River (CR) is the last major, undammed tributary flowing into the San Joaquin Delta, and fish from the CR (catfish and bass) tend to have elevated Hg concentrations relative to these fish in the Central Delta, despite generally lower sediment MeHg concentrations. Conversely, Frank's Tract (FT) is a flooded "island" in the Central Delta where fish Hg concentrations are typically lower than those in the CR, yet FT sediment MeHg concentrations are often higher (*22*, *23*). It is unclear why this apparent paradox in fish Hg concentrations exists. Here, we explored the relationship between DOC concentrations and bioconcentration of inorganic and methylmercury in four species of phytoplankton typical of those found in the SF Bay Delta. To measure Hg accumulation dynamics, we utilized ^{203}Hg added as either inorganic $^{203}HgCl_2$ or organic $CH_3{}^{203}HgCl$. Additionally, for the diatoms, we assessed passive versus active (or facilitated) uptake of both Hg(II) and MeHg via experiments with live and heat-killed cells and differentiated between cell wall or membrane bound and internalized Hg.

Materials and Methods

All experiments were conducted in sterile-filtered (0.2 μm) water collected from either the Cosumnes River (38°15.470′ N, 121°26.050′ W) or Frank's Tract (38°02.670′ N, 121°36.930′ W) in the SF Bay Delta system. Both water types were fresh (salinity = 0), but the CR water had lower DOC concentrations

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TABLE 1. Mean Concentrations (nmol g^{-1}) and Volume Concentration Factors (VCF) ± 1 SD for All Species at Steady State with Respect to Hg Partitioning, $n = 3$[a]

		Hg(II)			$CH_3Hg(II)$		
		water type			water type		
algal species	parameter	CR	FT	FT:CR	CR	FT	FT:CR
Cyclotella meneghiniana diatom, UTEX LB 2817 Vol: 287 μm^3; SA: 269 μm^2 SA:Vol: 0.94 μm^{-1} dry wt 162 pg	[nmol g^{-1}]	*23.2 ± 3.6	*9.7 ± 0.6	0.42	*92.4 ± 5.8	*197.5 ± 20.7	2.1
	VCF × 10^4	*0.86 ± 0.08	*0.43 ± 0.04	0.5	*14.5 ± 3.0	*37.8 ± 2.4	2.6
Chlamydomonas reinhardtii green alga, UTEX 90 Vol: 118 μm^3; SA: 114 μm^2 SA:Vol: 0.97 μm^{-1} dry wt 83 pg	[nmol g^{-1}]	41.8 ± 4.7	38.4 ± 0.4	0.9	*317.2 ± 60.9	*602.8 ± 126.2	1.9
	VCF × 10^4	2.08 ± 0.04	2.39 ± 0.21	1.2	31.0 ± 12.0	64.4 ± 31.3	2.1
Cryptomonas ozolini cryptophyte, UTEX LB 2194 Vol: 267 μm^3; SA: 224 μm^2 SA:Vol: 0.84 μm^{-1} dry wt. 145 pg	[nmol g^{-1}]	147.1 ± 12.6	166.0 ± 6.3	1.1	*176.3 ± 16.1	*450.5 ± 49.7	2.6
	VCF × 10^4	4.36 ± 0.20	4.95 ± 0.58	1.1	*12.7 ± 3.1	*31.7 ± 4.6	2.5
Synechocystis sp. cyanobacteria, UTEX 2470 Vol: 4.5 μm^3; SA: 13.3 μm^2 SA:Vol: 2.96 μm^{-1} dry wt 2.2 pg	[nmol g^{-1}]	93.5 ± 18.8	114.8 ± 22.5	1.2	*511.2 ± 37.1	*347.1 ± 20.3	0.7
	VCF × 10^4	1.74 ± 0.65	2.19 ± 0.91	1.3	144.6 ± 12.0	146.1 ± 57.3	1.0

[a] For each species, clonal designations, cell volume (Vol), surface area (SA), surface area:volume ratio (SA:Vol), and dry weight (dry wt) are shown. Steady state was at 48 h for *C. meneghiniana* and at 24 h for other species. Treatment effects were tested with one-way ANOVA with significant differences ($p \leq 0.05$) indicated (*).

(177 μM C ± 15.5) and lower pH (6.8) than FT water (280 μM C ± 40.1; pH = 7.9). DOC was measured on a Shimadzu TOC-5000 analyzer. Triplicate samples and reagent/ultrapure water blanks were sterile-filtered (0.2 μm) and acidified to pH ≤ 1 with HCl prior to analyses. We examined the diatom *Cyclotella meneghiniana*, the chlorophyte *Chlamydomonas reinhardtii*, the cryptomonad *Cryptomonas ozolini*, and the cyanobacterium *Synechocystis* sp. held in axenic clonal cultures. All are representative of the waters in the SF Bay Delta system (Müller-Solger, A. University of California—Davis. Personal communication, 2004). Characteristics of the cells are given in Table 1. Of particular interest for understanding metal sorption is the surface area to volume ratio, which was comparable for all three eukaryotic cells but 3-fold greater in the prokaryote. The cells were grown in complete WCL-1 medium (*24*) prior to experiments, were rinsed, were resuspended in WCL-1 without ethylenediaminetetraacetic acid (EDTA), and were grown for 7 days prior to Hg additions at 17 ± 0.5 °C on a 14:10 h light:dark cycle.

All experiments used the γ-emitting radioisotope ^{203}Hg to follow the dynamics of Hg accumulation and retention by the four species of phytoplankton. The ^{203}Hg used for all experiments was received as $^{203}HgCl_2$ in 1 M HCl with specific activities in different batches ranging from 153 to 325 kBq μg^{-1}. Radiolabeled monomethylmercurychloride (CH_3-^{203}HgCl) was synthesized from $^{203}Hg^{2+}$ as described elsewhere (*25*−*27*) and was stored in the dark in dilute (1×10^{-6} M) Optima grade HCl. Yields in the six syntheses of $CH_3{}^{203}Hg(II)$ carried out in our laboratory averaged 77.1% ± 7.8. All vessels used in the synthesis of MeHg, storage of stock solutions of Hg(II) and MeHg, and experimental flasks were put through a rigorous acid washing protocol with final drying in a trace metal clean, laminar flow hood equipped with a HEPA (0.2 μm) filter.

Experiments with Hg additions to low and high DOC waters and phytoplankton cells were amended with N, P, and Si at WCL-1 concentrations (*24*). Experimental algal cultures, held in 150 mL of the two waters without EDTA, received microliter additions of either $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$ in dilute HCl or neutral stock solutions, respectively, and dilute NaOH was added immediately to neutralize the acid additions. Depending on the specific activity of the available ^{203}Hg, radioactive additions to CR and FT water ranged from 17.3 to 30.7 kBq L^{-1} of ^{203}Hg, corresponding to ranges of 0.74−1.62 nM and 0.64−0.74 nM for Hg(II) and MeHg, respectively. Mercury concentrations in SF Bay Delta waters can range from 0.73 to 440 pM for Hg(II) and from 0.05 to 2.5 pM for MeHg (*21*). Initial algal biomasses ranged from 0.53 to 1.60 mg dry wt of cells L^{-1}, depending on the species. These initial biomasses allowed for subsequent growth in our medium for about 5−7 days for all species. Inocula came from log-phase cultures. Controls consisting of the two water types uninoculated with cells but with the identical ^{203}Hg additions were also examined to assess Hg binding to flask walls and Nuclepore membranes. All treatments were run in triplicate. The partitioning of the ^{203}Hg between dissolved and particulate phases and the growth of cells were determined periodically over time following protocols described elsewhere (*28*). Attempts were made to sample cultures aseptically during the course of the experiment, but no checks were made of bacterial growth in these cultures.

Radioactivity of ^{203}Hg(II) or $CH_3{}^{203}Hg^+$ was determined using an LKB Pharmacia Wallac 1282 Compugamma equipped with a well-type NaI(Tl) detector. Gamma emissions of ^{203}Hg were assayed at 279 keV and counting times were 10 min to yield propagated counting errors ≤5%. All samples were counted with appropriate blanks and standards to correct for decay and background radioactivity. Radioactive counts were normalized on a per cell basis after subtracting the sorption of ^{203}Hg to filters (from controls), typically a 6−20% correction for Hg^{2+} experiments and ≤10% for MeHg experiments. These counts were used to determine volume concentration factors (VCFs) of Hg(II) or MeHg in cells when the enrichment of radioisotope in algal cells relative to ambient water remained constant, expressed on a volume/volume basis (*28*).

Even though cells continued to divide, a steady state was reached within 24−48 h for mercury VCFs. This measure of mercury enrichment is defined as the amount of mercury per μm^3 cell divided by amount of mercury per μm^3 in the dissolved phase in ambient water. VCFs and Kds (metal enrichment normalized to dry wt) effectively compare the extent to which different metals get enriched by different algal species (*29*); because of varying densities among algal species with different cell wall characteristics, we suggest that VCFs allow for more appropriate comparisons among species.

To delineate between active accumulation (defined as requiring cells to expend energy) of Hg(II) and MeHg from passive sorption, we contrasted ^{203}Hg activity in heat-killed diatom cells and in live cells. Prior to determining ^{203}Hg accumulation, preliminary experiments with microscopic and fluorometric validation determined that submerging culture flasks in a 50 °C water bath for 10 min killed *Cyclotella meneghiniana* cells without rupturing or damaging cell integrity. Water held in flasks (three replicates/treatment) for each water type was inoculated with live *C. meneghiniana*

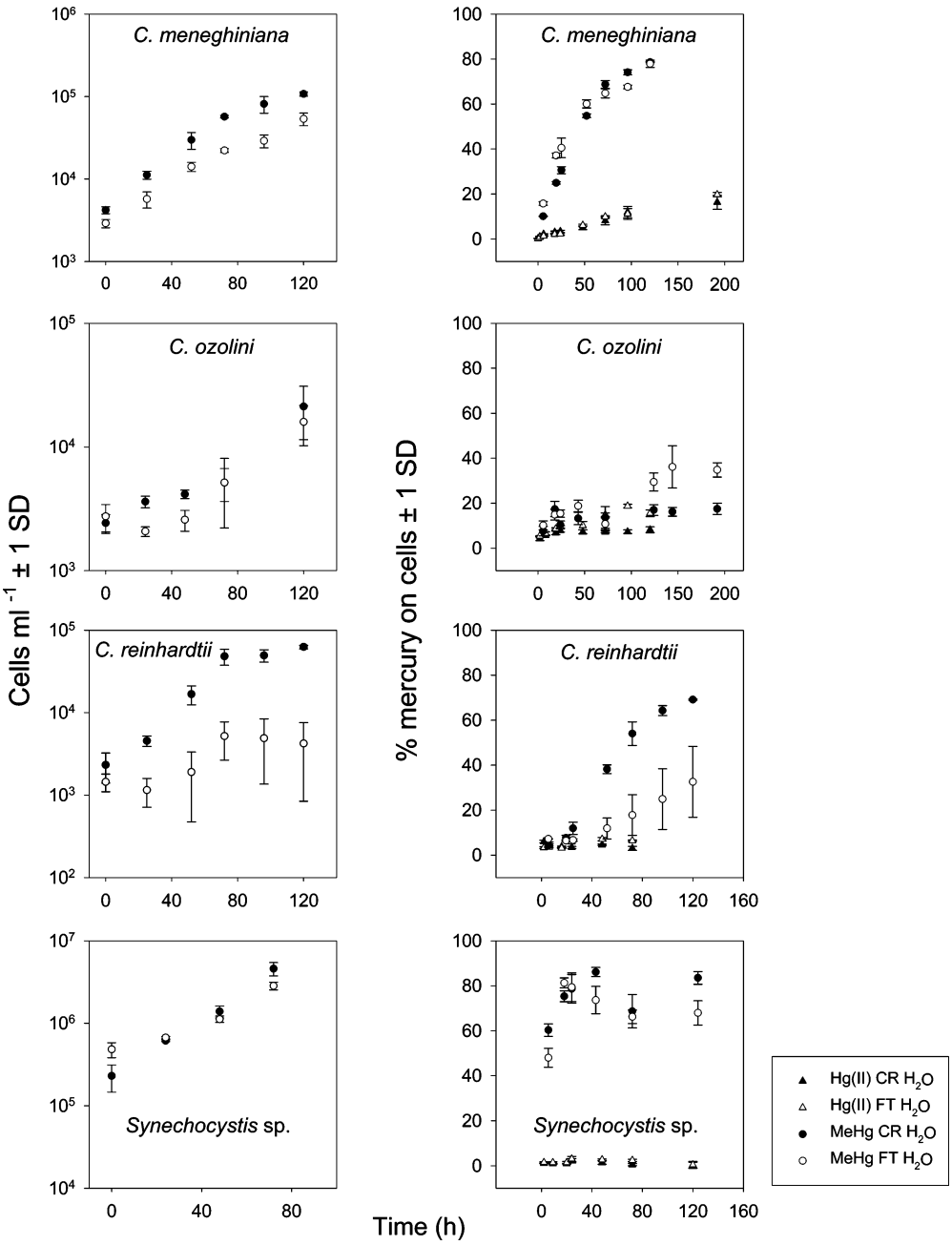

FIGURE 1. Cell densities (cells mL^{-1}) in both low DOC, Cosumnes River (CR) water (●) and high DOC Frank's Tract (FT) water (○) for four freshwater algal species with time (left panels). Data presented are from the MeHg exposure experiments. The percentage of Hg(II) and MeHg on cells in both CR (low DOC) and FT (high DOC) waters in four freshwater algal species with time (right panels). Values are means ± 1 standard deviation (SD), $n = 3$ for each plot.

at 2.6−3.5 mg cells L^{-1}. For each water type, half the flasks were submerged in a hot water bath to kill cells and ^{203}Hg additions were made immediately afterward to both live and dead cultures as either Hg(II) or MeHg; cell growth and uptake of ^{203}Hg were then followed over a 48-h period.

An additional experiment with live and dead *C. meneghiniana* cells in the low DOC, CR water was conducted to compare the cytological distribution of Hg that accumulated in the diatoms. Since cellular uptake of MeHg may occur via a methionine uptake pathway (*30*), we also examined the influence of an equimolar (0.75 nM) concentration of L-methionine on the uptake of Hg(II) and MeHg in living and dead cells. After cellular uptake of MeHg or Hg(II), with or without added methionine, reached steady state, about 6×10^5 cells were resuspended in 10 mL of CR water without Hg and ruptured by sonication for 10 min. Cytoplasmic contents were separated from cell wall and membrane fractions by centrifugation (10 min at 21 000*g*), and the proportion of total cellular ^{203}Hg in each of the two fractions was determined (*31*), taking into consideration Hg desorption into the Hg-free water.

Results

Mercury added to the two culture waters as either inorganic Hg(II) or MeHg was accumulated by each of the four phytoplankton species. As cells grew over time, as much as 70−80% of the added MeHg was removed from the aqueous phase and became associated with cells (Figure 1). Generally, the increase in particulate Hg mirrored the growth of cells (i.e., particle surfaces), yielding a steady-state relationship

between Hg enrichment in cells relative to ambient water. For each of the four algal species, the partitioning of inorganic Hg(II) to live algal cells was always lower than that of MeHg.

At steady state with respect to volume-normalized Hg partitioning between dissolved and particulate phases, the concentrations of Hg(II) in the four algal species ranged from 23 to 147 nmol g^{-1} in the low DOC water and from 10 to 166 nmol g^{-1} in high DOC water; MeHg levels ranged from 92 to 511 nmol g^{-1} in low DOC water and from 198 to 603 nmol g^{-1} in high DOC water (Table 1). VCFs of Hg(II) ranged from 0.86 to 4.36 $\times$ 10^4 in low DOC water and from 0.43 to 4.95 $\times$ 10^4 in high DOC water (Table 1). The VCFs differed significantly between water types only for *C. meneghiniana* where values were 2-fold greater in low DOC water (Table 1). The VCFs of MeHg ranged from 12.7 to 144.6 $\times$ 10^4 in low DOC water and from 31.7 to 146.1 $\times$ 10^4 in high DOC water, with values in high DOC water being 2−2.6 times those in low DOC water for the eukaryotes (Table 1). No differences were noted for MeHg VCFs in the prokaryote (*Synechocystis* sp.) between water types. Relative differences of organic and inorganic Hg bioconcentration varied with species and water type. Within each species, the VCFs of MeHg were 3−6 fold greater than Hg(II) VCFs for *C. ozolini*, 8−16 fold greater for *C. reinhardtii*, 17−88 fold greater for *C. meneghiniana*, and 67−83 fold greater for *Synechocystis* sp. (Table 1). No significant correlation was noted between the VCF of Hg(II) and the surface area:volume ratios for any of the four species ($r^2 = 0.021$, $p > 0.73$), however, a positive correlation ($r^2 = 0.922$, $p < 0.001$) was noted for MeHg VCFs and cell surface area:volume ratios, with the smallest cells displaying the highest VCFs. It is recognized that this high correlation is influenced by two very different sets of cells with different surface:volume ratios; cells with intermediate ratios were not considered in our study.

In the experiments comparing the bioaccumulation of Hg in living and dead diatom cells, the sorption pattern of Hg(II) over time reflected cell growth (none in dead cells), and VCFs of Hg(II) in living and dead diatom cells, in both water types, were comparable (Figure 2). However, the VCFs of MeHg in living diatoms were significantly greater (e.g., at $t = 48$ h contrasts of live versus dead cells via one-way ANOVA for CR H_2O: $p < 0.018$, $F_{1,5} = 15.25$; for FT H_2O: $p < 0.0003$, $F_{1,5} = 146.8$) than those in dead cells in both water types, with differences most apparent in low DOC water (Figure 2). The proportion of MeHg associated with diatom cytoplasm ranged between 59 and 64% for live cells, but only 9−16% of the Hg(II) crossed cell membranes to enter cell cytoplasm (Table 2). In all treatments, there was negligible accumulation of Hg in the cytoplasm of dead diatoms. No significant effect of methionine on Hg accumulation was evident (i.e., no effects on VCFs or cytological distribution of Hg).

Discussion

The cellular uptake of inorganic and methylmercury over time increased with cell growth, reflecting the increased particle surface to which Hg could bind. Mercury accumulation among the four species was comparable to previously reported values for Hg(II) (*4*, *5*) and for eukaryotic cells for MeHg (*5*, *32*). While some studies have shown higher partition coefficients (K_d) for inorganic Hg in natural seston (log K_d = 4.1−5.7) than for MeHg (log K_d = 2.2−5.3) (*33*−*35*), we found higher K_d's for MeHg (log K_d = 5.1−6.2) than for Hg(II) (log K_d = 3.6−4.7) with the cultured cells. These differences may in part be due to the higher reactivity of inorganic Hg than of MeHg for abiotic particle surfaces, which are not generally separated from phytoplankton in natural seston. Consistent with this idea, we found that binding of Hg(II) to the glass walls of our experimental flasks containing uninoculated filtered water (i.e., controls) was about 9 times greater after 48-h incubation (27% vs 3%) than that of MeHg.

In natural surface waters, the proportion of MeHg bound to colloidal material between 0.7 μm and 10 kDa is typically greater than that in the "truly dissolved" (<10 kDa) phase (*36*, *37*). We did not measure the colloidal association of either mercury species in our experiments. It remains unclear how colloidal material or the quality of DOC (see below) in the SF Bay Delta affects Hg uptake by phytoplankton cells.

That the concentration factors of Hg(II) were comparable for the four algal species suggests that the cell wall constituents of these cells did not affect sorption of the Hg. Thus, diatoms with silica frustules, chlorophytes with cellulose walls, and cyanobacteria with peptidoglycan walls all exhibited comparable enrichment of Hg(II). Even the greater surface-to-volume ratios of the smaller cells had no appreciable effects on this enrichment. Many other metals similarly display small differences in their bioconcentration in phytoplankton of diverse composition and cell wall features (*38*). It has been speculated that such similarities among species might be attributable to the binding of metals to organic films coating all cells in natural waters (*29*); since all cells have the same organic films coating them, the metal encounters the same surface regardless of the cell type. The binding of Hg(II) to dead diatoms and the constant VCFs across species noted here suggest that the association of inorganic Hg to algal cells proceeds passively, also consistent with previous studies with mercury and other metals (*8*, *39*).

In contrast with Hg(II), the uptake of MeHg appears to be, at least in part, metabolically controlled, since accumulation in live diatoms was up to an order of magnitude greater than in dead cells (normalized to biomass). The lack of a competitive effect of methionine on Hg uptake in *C. meneghiniana* suggests either a lack of saturation of the methionine uptake pathway or that uptake of Hg(II) and MeHg do not proceed through this pathway. Among the eukaryotes examined, no appreciable differences were found in MeHg VCFs, but uptake of MeHg by the prokaryote was significantly greater within a given water type (up to 10 fold) and was consistent with their higher surface-to-volume ratios. It is possible that the algal cells actively accumulated organic compounds such as amino acids to which MeHg was bound, thus accounting for the active uptake component of MeHg and consistent with its differential uptake in the two waters. Our data corroborate previous research supportive of active processes involved in MeHg accumulation in freshwater phytoplankton (*4*, *19*, *32*). The finding that MeHg enrichment was highest in the picoplanktonic cyanobacterium (*Synechocystis* sp.) is in contrast to these earlier studies that used much larger cyanobacterial cells (cell volume = 212 μm^3) (*19*, *32*).

Our data on the cytological distribution of Hg(II) and MeHg in living *C. meneghiniana* cells (9−16% and 59−64%, respectively) are directly comparable to results of an earlier study with a marine diatom (*Thalassiosira weissflogii*) in which 9% of inorganic and 63% of methylmercury were found in the cytoplasm (*5*). Methylmercury and to a lesser extent inorganic mercury penetrate into the cytoplasm of algal cells as this is directly linked to the efficiency with which they are assimilated into herbivores (*5*, *31*) and build up in aquatic food chains. Once in the cytoplasm of some cells, most notably the chlorophyte *Dunaliella tertiolecta*, mercury can be detoxified by being sequestered in sulfide precipitates within the cell (*8*, *40*). Metals in such precipitates are less efficiently assimilated in herbivores feeding on these cells (*41*, *42*).

The bioaccumulation of inorganic Hg(II) was generally comparable in low and high DOC water; thus, any differences in chloride concentrations between the waters, DOC concentrations, or DOC composition appeared to have no consistent effect on uptake of Hg(II). However, the bioaccumulation of MeHg was consistently greater in high DOC water for all species, presumably because of speciation

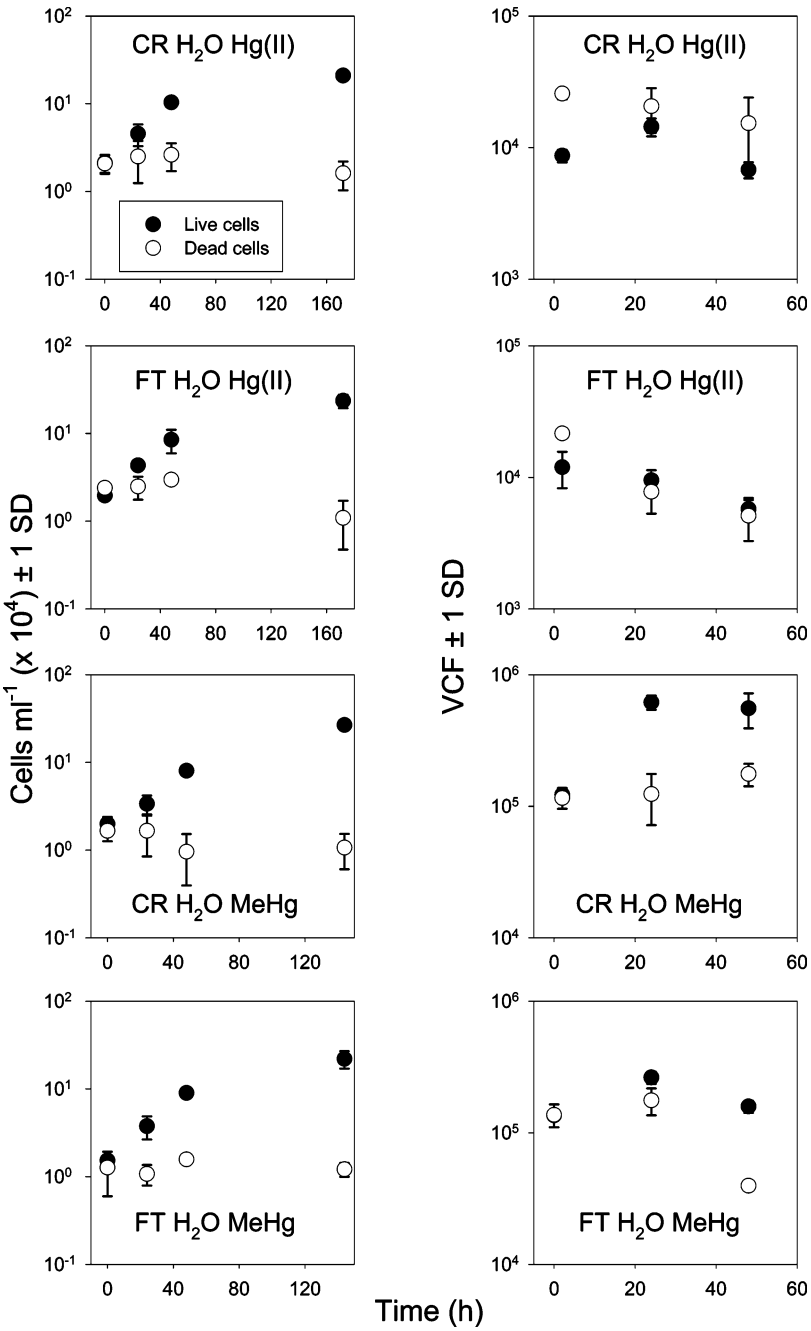

FIGURE 2. Cell densities (cells $mL^{-1} \times 10^4$) for live (●) and dead (○) *Cyclotella meneghiniana* cells in low DOC, Cosumnes River (CR) and high DOC Frank's Tract (FT) water with time (left panels). Volume concentration factors (VCFs) for *C. meneghiniana* for Hg(II) and MeHg in CR (low DOC) and FT (high DOC) water with time (right panels). Values are means ± 1 standard deviation (SD), $n = 3$ for each plot.

differences in the two water types, possibly related to differences in the dissolved organic compounds present.

Here, we consider two possible explanations that may account for differences in MeHg bioavailability but not in Hg(II) bioavailability: (1) the proportion of the added CH_3HgCl that remained as the neutral chloride complex was greater in high DOC water and (2) the concentration and composition of the DOC in FT water enhanced cellular uptake of MeHg. First, we consider the influence of chloro-complexation of mercury on its bioavailability for phytoplankton.

The speciation and stability of MeHg complexes in surface waters are dependent upon several parameters (*43*), including pH and the concentration of chloride ion (pCl, calculated as $-\log[Cl^-](M)$) (*5*, *44*). Porewater pCl concentrations in high DOC, FT water are 2.41 ± 0.09 and 3.70 ± 0.09 in low DOC, CR water (means ± 1 SD; $n = 22$, Marvin-DiPasquale, United States Geological Survey, Menlo Park, CA, unpublished work, 2006), and it is reasonable to expect that the porewater chloride ion concentrations are representative of the overlying waters used in our experiments. At the pH (7.3−7.9) and chloride concentration measured in the high DOC water, thermodynamic stability constants predict approximately 50% of the added CH_3HgCl to remain as the neutral chloride complex with 50% shifting to the CH_3HgOH complex typical of fresh, surface waters with low chloride concentrations (*5*, *44*, *45*). The speciation of the added organic mercury in the low DOC water at lower pH (6.4−6.9) and >10 fold lower Cl^- concentration was probably dominated by the hydroxo-complex.

TABLE 2. Cellular Partitioning of Mercury between Cytoplasm and Cell Walls and Membranes in the Diatom *Cyclotella meneghiniana* for Live and Dead Cells in the Presence or Absence of Added Methionine[a]

treatment	% of Hg in cell walls and membranes	% of Hg in cytoplasm
live cells exposed to MeHg	36.0	64.0
dead cells exposed to MeHg	95.9	4.1
live cells exposed to MeHg + methionine	40.8	59.2
dead cells exposed to MeHg + methionine	100	0
live cells exposed to Hg(II)	84.5	15.5
dead cells exposed to Hg(II)	99.8	0.2
live cells exposed to Hg(II) + methionine	91.1	8.9
dead cells exposed to Hg(II) + methionine	100	0

[a] No significant effects of methionine on Hg VCFs were noted.

The greater proportion of the neutral methylmercury chloride in the high DOC water likely increased the accumulation of MeHg by the cells because of its greater lipid solubility than that of CH_3HgOH. The ability of MeHg complexes to cross cell membranes can be estimated by octanol−water partition coefficients (K_{ow}). The log K_{ow} for CH_3HgCl is 1.7 and for CH_3HgOH it is only 0.07 (*45*), so appreciable chloro-complexation of the MeHg remaining in the high DOC treatments would result in increased VCFs for cells in that water. The lack of consistent, significant differences in the accumulation of inorganic Hg(II) by phytoplankton between the two waters suggests that Cl^- concentrations did not appreciably alter Hg(II) speciation and thus K_d values. At the chloride and pH ranges for the two waters, thermodynamic predictions are that added $^{203}HgCl_2$ was converted to both $^{203}Hg(OH)_2$ and $^{203}HgOHCl$ (*5*, *44*). The log K_{ow} values for $Hg(OH)_2$ and HgOHCl, 0.05 and 1.20 (*5*), respectively, are substantially lower than that for $HgCl_2$ (3.33). The relatively high K_{ow} values for $HgCl_2$ and HgOHCl exceed or are similar to those for the organic, MeHg species (0.07−1.7), and the presence of either HgOHCl or $HgCl_2$ should have produced VCFs exceeding those for MeHg. However, since the VCFs for all phytoplankton species were always 1−2 orders of magnitude lower for Hg(II) than for MeHg, it appears that these inorganic chloro-complexes did not significantly influence uptake. The speciation modeling and patterns of algal uptake indicate that $Hg(OH)_2$ was the dominant form of inorganic Hg(II) in our experiments, typical of oxygenated freshwaters with pCl values between 2.0 and 4.5 and pH values between 6.0 and 8.0.

The bioavailability of organic complexes of inorganic mercury and methylmercury may also help account for our observations of their algal uptake in the two water types. Almost 95% of the oxidized inorganic Hg can be bound to dissolved organic matter (*46*), and much of the Hg in our experiments may well have been bound to dissolved organic matter. However, since no significant differences in Hg(II) accumulation between the high (FT) and low (CR) DOC waters were evident for any phytoplankton species, it appears there were no appreciable differences in organic complexation of Hg(II) between the two waters. Similar conclusions were drawn for Hg(II) bioavailability in coastal waters (*12*). The higher concentrations of DOC in FT water than in CR water (1.6-fold different) may have accounted for the 2.4-fold greater accumulation of MeHg in eukaryotic phytoplankton in FT water. In a study with different freshwater phytoplankton species, the presence of humic substances was found to alter the permeability of cell membranes resulting in enhanced metal uptake, although these effects were most apparent from pH 4 to 5.7 (*14*, *47*, *48*). It is possible that the DOC in FT water enhanced membrane permeability to MeHg but not inorganic mercury, although we have not quantified the effects of this material on membrane permeability in the phytoplankton in this study. Studies are starting to characterize the dissolved organic matter in the Delta region (*49*), but the exact composition of the organic compounds present in Frank's Tract and Cosumnes River waters remains unknown, as does the extent to which they influence membrane permeability to mercury and other metals.

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